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SKELETAL CHANGES AFFECTING THE NERVOUS SYSTEM PRODUCED IN YOUNG DOGS BY DIETS DEFICIENT IN VITAMIN A

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I. Introduction

The production under controlled conditions of tissues abnormal in structure or function, or in both, and the prevention of the abnormalities by defined alterations in these conditions have sometimes led to the accession of knowledge both to the physiologist and pathologist. The present paper deals with an instance of this type of experiment.

In previous publications detailed descriptions have been given of the widespread degeneration of the central and peripheral nervous systems, especially of young animals brought up on diets deficient in vitamin A and carotene and rich in cereals [Mellanby, 1926, 1931, 1933, 1934, 1935]. Although these degenerative changes were thought at first to be directly due to the destructive effect of vitamin A deficiency on the nervous system, evidence was subsequently forthcoming to suggest that they were indirectly produced and were really due to bone overgrowth in the vicinity of the affected nerves and nerve cells and to the pressure effects resulting therefrom. In particular, a study of the eighth nerve and the labyrinthine capsule [Mellanby, 1938] demonstrated that a dietary deficiency of vitamin A resulted in bony overgrowth of the periosteal layer of the labyrinthine capsule, especially of that part surrounding the internal modiolus, and the evidence strongly suggested that the pressure due to this overgrowth was responsible for the nerve degeneration. At a later date, evidence was also given of bony overgrowth surrounding the optic and trigeminal nerves and of other bones in close proximity to the brain and spinal cord [Mellanby, 1939*a*, *b*]. Although it cannot yet be claimed that this sequence of events fully explains all the widespread

nerve degeneration that occurs in these animals, the evidence accumulating continues to support this view.

All or nearly all bones in the body are affected by these vitamin A deficient diets, but this report will deal mainly with the gross changes in the bones of the skull and of the vertebral column and the effect these changes have on the nervous system. It is not proposed to discuss here in detail the complicated bone changes which damage individual cranial nerves. A short account will, however, be given of the destructive effects on the spinal nerves produced by bony overgrowth of the vertebrae.

As regards the wider question why bones undergo these interesting changes and why some bones, and even parts of bones, are more affected than others in vitamin A deficiency, but little can be said at present. At first it appeared as if bones developing from cartilage were more liable to be affected than membranous bones, but closer examination revealed that this hypothesis had scanty basis. Probably the essential cause of these differences is the varying rates of growth of individual bones or of regions of a given bone. This explanation may also account for the relative ease with which bone overgrowth is produced by these diets in young, as compared with adult, animals. If, for instance, the special diets are started when puppies are 6–9 weeks of age, the animals' in-co-ordination of movement and other signs of abnormal behaviour and appearance develop 2–4 months later, and after 4 months the abnormalities may be very great. In fully grown animals, on the other hand, although vitamin A deficiency affects bone structure, the action is slow and many months or even a year or more may be required to produce noticeable changes.

Rate of growth, however, is not the only factor in deciding time of onset and extent of the bone changes. Litters of puppies, whose storage of vitamin A in the liver and elsewhere is great because of the maternal and pre-experimental diets being rich in this substance, are much longer resistant to vitamin A deficiency than litters whose stores are small. It is clear, therefore, that susceptibility of animals to these experimental diets is apt to vary greatly according to a number of factors and especially to their rate of growth, age and stores of vitamin A at the beginning of the feeding period.

From the point of view of the physiologist, the main question to be answered concerns the part played by vitamin A in normal bone growth. This question cannot be fully answered yet, but an indication of a relationship is given in the observations described below on the osteoblastic and osteoclastic changes produced in bone by vitamin A deficiency.

That such reactions may afford the fundamental explanation of the widespread changes in bone is in keeping with the importance of age and rate of growth in determining the rate of development of the bone hyperplasia, for it is in the early stages of life and in places of more rapid growth that osteoblasts and osteoclasts are more numerous and active.

It may be well to add that the effects of vitamin A deficiency here described must be considered in relation to the basal diets given. There is indeed evidence that some modifications of the basal diet, even in the absence of vitamin A, also affect the results, at least in degree if not in kind. This is not surprising in view of the numerous known dietetic factors which influence bone structure and bone growth. In particular, the addition of calcium salts and the presence or absence of vitamin D modify the bone overgrowth in the absence of vitamin A. The results obtained under the conditions described do, however, show that bone growth is affected by vitamin A and that these changes may have notable effects on nerve function and animal behaviour.

II. Experimental methods

Litters of puppies from 7 to 10 weeks old were fed on basal diets of the following type: separated milk powder 20 g., cereal (usually white bread) 100–300 g., lean meat 15–20 g., yeast 3–12 g., peanut or olive oil 10 ml., orange or lemon juice 6 ml., sodium chloride 1–2 g., and irradiated ergosterol (vitamin D_2) 1000–2000 international units. This diet, although made up of natural foodstuffs, with the exception of vitamin D_2, is deficient in vitamin A and carotene, there being only a small amount of vitamin A in the lean meat and a trace of carotene in the cereal. The diet is otherwise good, although its calcium content may not be sufficient to allow optimal calcification of bones when growth is very rapid. In such cases a certain degree of osteoporosis, but never rickets, may develop even when vitamin A is present; otherwise the diet allows good growth and healthy development, except for the abnormal changes produced by vitamin A deficiency. In each litter one or more puppies were given an additional supply of vitamin A or carotene; the former usually as mammalian liver fat preparation, but sometimes as cod-liver oil, and the latter as carotene in cabbage or as the pure substance. The form in which vitamin A or carotene is given does not appear to matter, and, so long as sufficient is absorbed from the alimentary canal, normal bones result and the nervous system does not show the characteristic degenerative changes.

Preparation of tissues for examination

At the end of each experiment, the tissues were prepared for histological examination. In order to reduce shrinkage of soft tissues to a minimum, an intra-arterial fixation method was used and in most cases serial sections of the bones were made. The methods of preparation used were largely in accordance with Wittmaack's technique and his solution was generally used for intra-arterial fixation.

III. Bone overgrowth and its effects on the nervous system

A. *Skull, brain and cranial nerves*

A comparison of mesial sagittal surfaces of the skulls of two litter mates, one of which had a diet containing much vitamin A and the other a diet deficient in the vitamin, shows some of the regions which are especially subject to bone overgrowth. Diagrams of the cut surfaces are given in Text-figs. 1*a* and 1*b*; Text-fig. 1*a* represents the mesial sagittal section of the skull and brain of a dog brought up on a diet containing much vitamin A (+A dog), Text-fig. 1*b* of a dog whose diet was otherwise the same except that it was deficient in this vitamin (−A dog). (For the sake of brevity the terms +A and −A dog (or animal) will be used to denote dogs brought up on diets containing, or deficient in, vitamin A respectively.)

It will be noted that the brain of the animal maintained on a diet deficient in vitamin A is more tightly packed into the cranial cavity than that of its litter mate receiving vitamin A. It will also be seen that the bones showing the most overgrowth are those surrounding the cerebellum, medulla oblongata and pons. In particular, all parts of the occipital bone, both above and below the foramen magnum, are greatly enlarged (Text-fig. 1*b*) as compared with the normal (Text-fig. 1*a*). Passing forward from the supra-occipital bone, the parietal bone is also much thickened in its posterior part, but this thickening becomes less as it approaches the frontal bone. Similarly, at the base of the skull, passing forward from the hypertrophied basi-occipital bone, the enlargement of the basal portion of the sphenoid, although definite, is not so great as in the occipital bone.

The effect of this overgrowth of bone at the posterior end of the skull is to press on the cerebellum and medulla oblongata and alter their shape. The cerebellum is flattened on its dorsal surface and its posterior surface is indented with the thickened occipital bone just above the foramen magnum. In the particular skull illustrated in Text-fig. 1*b*, the tentorium cerebelli has become calcified and a wedge of bone separates

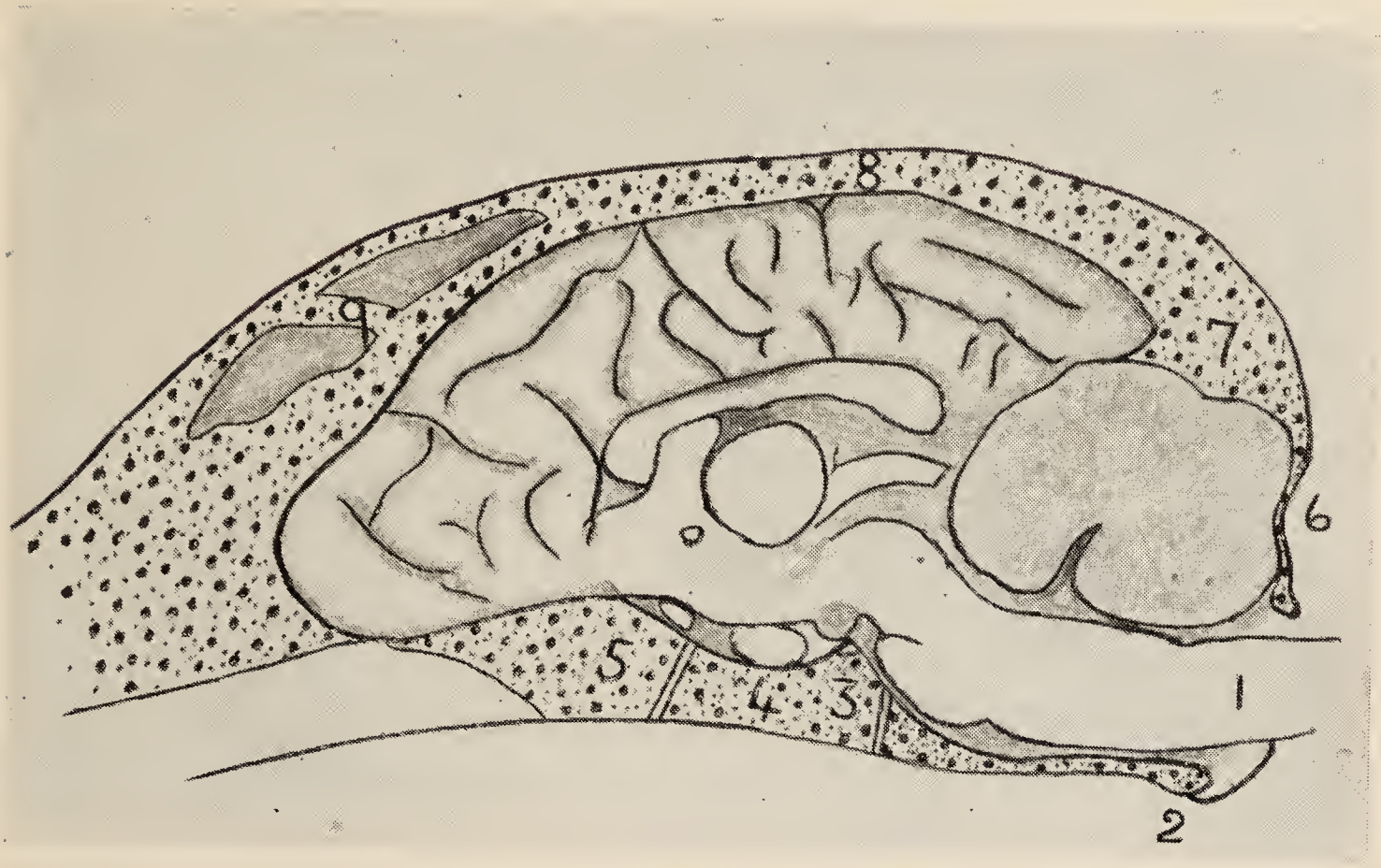

Fig. 1*a*.

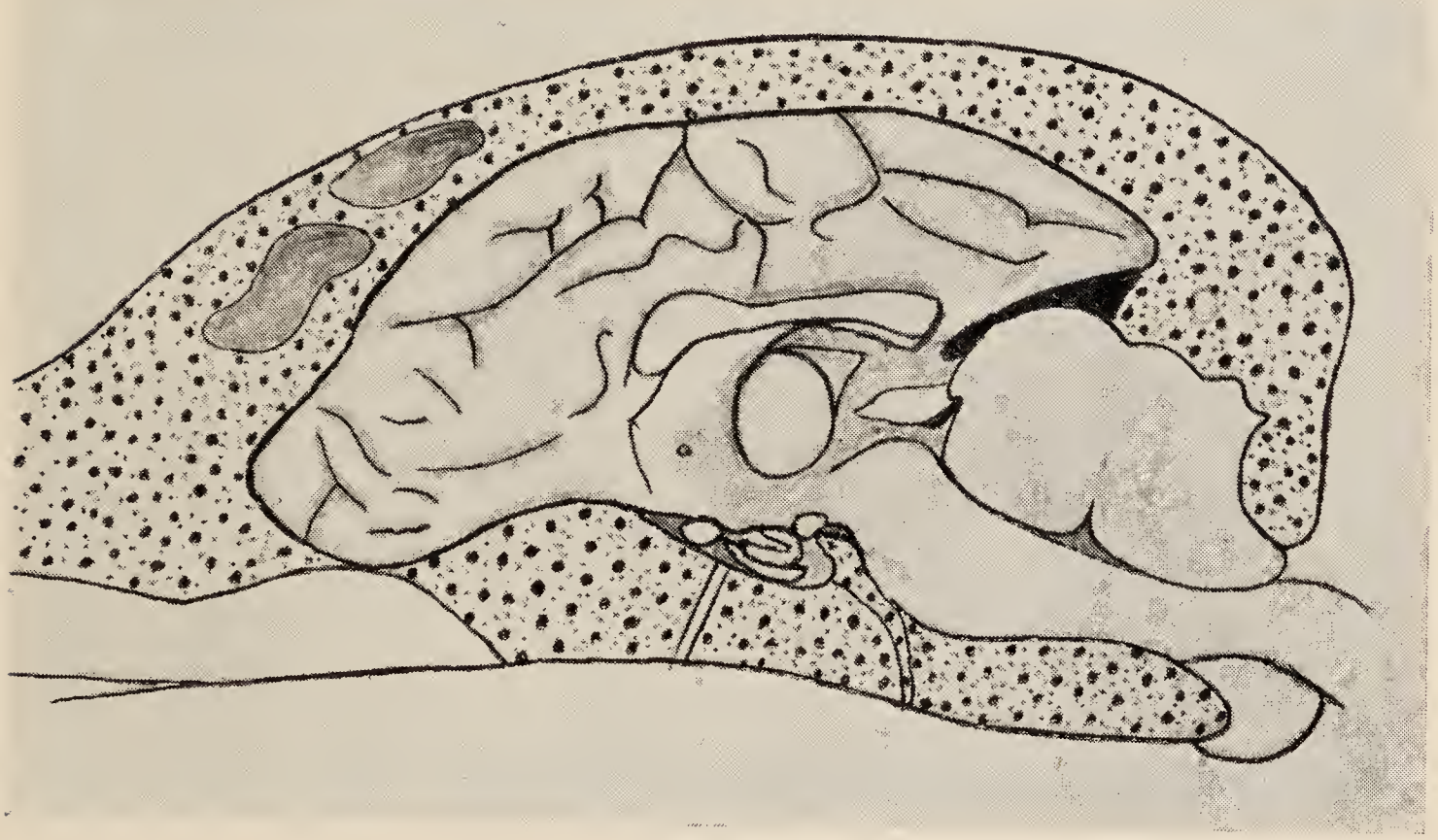

Fig. 1*b*.

Text-figs. 1*a* and 1*b*. Mesial sagittal sections of skulls of +A (*a*) and −A (*b*) dogs (bones stippled). Note great increase in thickness of bones forming base of skull, supra-occipital and to a less extent other bones in 1*b* as compared with those of 1*a*; also compression of medulla and cerebellum and pushing back of the posterior part of the cerebellum into the foramen magnum in 1*b*. 1, foramen magnum; 2, basi-occipital; 3, posterior clinoid process; 4, basi-sphenoid; 5, anterior clinoid process; 6, supra-occipital; 7, occiput; 8, parietal; 9, frontal. The calcified tentorium cerebelli is marked black in text-fig. 1*b*.

the occipital lobe of the cerebrum from the anterior dorsal portion of the cerebellum. Text-fig. 1*b* also shows that the posterior ventral portion of the cerebellum is pressed backwards through the foramen magnum between the occipital bone and the dorsal surface of the medulla oblongata, a space which is normally part of the cisterna magna. This intrusion of the cerebellum into the cisterna magna can be better appreciated in Pl. I, fig. 2*b* (compare Pl. I, fig. 2*a*, the cisterna magna of +A dog).

The medulla oblongata, instead of being cylindrical in that part just ventral to the cerebellum as in Text-fig. 1*a* (+A), is compressed in Text-fig. 1*b*, and the 4th ventricle and the aqueduct of Sylvius are narrowed and reduced in capacity. The overgrowth of the occipital bone surrounding the foramen magnum lessens the area of the aperture through which the posterior end of the medulla passes and must press on this part of the nervous system. Actual narrowing of the dorsi-ventral cross-section of the medulla at this point can be seen by comparing Text-fig. 1*b* (−A) with Text-fig. 1*a* (+A).

In Text-fig. 1*b* there is also a change in the posterior clinoid process at the posterior end of the sella turcica. It is enlarged dorsally and bent forward at its free end. In some experiments this overgrowth has been so great that it has compressed the pituitary body.

While the above are some of the more obvious defects seen in the sagittal sections, removal of the brain reveals other bone overgrowths. Most prominent is the hypertrophy of the petrous portion of the temporal bone. The petrous ridge is normally a fine crest of compact bone, but in −A animals it is a bulbous mass of cancellous bone with a very thin covering of compact bone. This overgrowth must further reduce the space available for that part of the brain contained in the posterior fossa and thereby increase the pressure on the pons, the cerebellum, medulla oblongata and nerves closely related to them. Reference has been made in previous papers to the hyperplasia of this portion of the temporal bone in relation to the 8th nerve and an account has been given of its stretching effect on the nerve and, indeed, of the complete destruction of the nerve in some cases by the occlusion of the passage from the labyrinth to the brain [Mellanby, 1938]. The enlargement of the petrous ridge also partially closes the 5th nerve foramen and compresses this nerve and the gasserian ganglion. When the nerve and ganglion are dissected out, they show a definite constriction where they have passed under and been compressed by the petrous ridge. The increased size of the temporal bone in this position also stretches and pinches the 7th nerve. The 9th, 10th and 11th nerves are trapped between the enlarged temporal and basi-

occipital bones in their passage through the jugular foramen. Examination of serial sections through the jugular foramen of −A and +A animals suggests that the glossopharyngeal nerve escapes destructive pressure change, while the vagus and its ganglion and the spinal accessory nerve are probably compressed. The 12th nerve passes directly through the thickened basi-occipital bone; it is elongated in this part of its course and may be compressed. Thus, it will be seen that all the nerves from the 5th to the 12th, with the exception of the 6th, are liable to be directly affected by compression and stretching owing to the overgrowth of the temporal and other bones forming the posterior cranial fossa.

Passing forward in the brain to the middle cranial fossa, the direct pressure of the bone is less, since neither the sphenoid nor the parietal bone shows the great thickening noted in the temporal and occipital bones.

While the bone changes in the neighbourhood of the anterior cranial fossa are not large, especially as compared with those of the posterior cranial fossa, they may nevertheless damage the optic nerve. This is affected in two ways. In the first place, the anterior clinoid process may be enlarged and thus bring about a lateral displacement of the optic foramen which tends to stretch and to bend the nerve. In addition, the optic foramen is narrowed by bone overgrowth and the nerve may be compressed. In advanced cases these effects can be readily seen when the optic nerve is removed from the skull: it is constricted in that part which passes through the optic foramen and it is tortuous and lengthened by the lateral displacement of the foramen. Blindness in calves due to constriction of the optic nerve has been described by Moore, Huffman and Duncan [1935]; also Moore [1939].

There is also enlargement of the cribriform plate, again with a superabundance of cancellous tissue at the expense of its compact bony covering. In the dog the cribriform plate forms a large part of the wall of the anterior cranial fossa. Although the cribriform plate in −A dogs does not appear very abnormal to the naked eye, it will be shown in a later publication that the overgrowth may be disastrous to the branches of the olfactory nerve which pierce the plate, for in some cases many of the nerve fibres are undoubtedly compressed and destroyed. While it is true in general that overgrowth in the frontal bone is small as compared with that in some other bones in the skull, thickening of this bone may sometimes take place at the expense of the frontal sinus, which is reduced in size. Beneath and slightly posterior to this sinus the frontal bone may occasionally be seen bulging into the anterior cranial fossa, whereas in the normal dog this area is usually concave.

Only a brief reference to the condition of the cranial nerves has been given here. Serial sections through the skull bones and the nerves have been made and will be described elsewhere. These confirm that in many cases there is actual compression and stretching of the nerves by bone overgrowth and this overgrowth may well account for most of the degenerative changes in the nerves.

The following measurements of skulls illustrate some of the main overgrowths which directly affect the brain. They represent measurements of comparable regions of the skull bones of two dogs (litter mates) on vitamin A rich and vitamin A deficient diets respectively. (See Text-figs. 1*a* and 1*b*.)

Bone	+Vitamin A dog mm.	−Vitamin A dog mm.
Foramen magnum (dorsi-ventral) (1)	15·8	12·5
Basi-occipital (2)	4·2	8·5
Posterior clinoid process (3)	9·0	12·0
Basi-sphenoid (4)	3·8	6·0
Anterior-clinoid process (5)	10·0	13·0
Supra-occipital (6)	3·0	6·9
Occiput (7)	11·5	18·0
Parietal (8)	4·0	6·0
Frontal (9)	5·0	6·0

These figures again prove that with a deficiency of vitamin A there is great hypertrophy of the bones surrounding the posterior fossa and a smaller overgrowth of those anterior to this fossa. The reduction in the size of the foramen magnum is also apparent.

B. *Changes in intracranial pressure*

That the pressure on the brain is increased in certain places is undoubted for, as shown above, at least in the posterior cranial fossa, the brain is actually deformed by the bone overgrowth. The question arises as to whether there is a general increase in intra-cranial pressure and, if so, whether it is equal in all parts. In this connexion a number of points must be considered. In the first place, the overgrowth is proportionately much greater in the bones surrounding the hinder part of the brain. If there were no obstruction between the posterior and middle cranial fossa, the increased pressure of the hypertrophied bones on the pons, cerebellum and medulla oblongata would undoubtedly be spread over the whole brain. This spread, however, is probably hampered on the dorsal aspect by the tentorium cerebelli; in some of the −A animals this obstruction is more effective because, instead of being membranous as in normal animals, the tentorium is partially calcified. The cerebrospinal fluid, however, will presumably tend to distribute the pressure via the basa

there is often a large increase of pressure in the cerebrospinal fluid of −A dogs and that, associated with this, there is

(1) compression and reduction in size of the 4th ventricle, cisterna magna and other cisternae at the base of the brain;

(2) a condition of internal hydrocephalus with expansion of the lateral and 3rd ventricles.

Whether there is ever complete occlusion of the aqueduct of Sylvius and the foramina of Luschka, either temporarily or permanently, is not known, but the evidence is rather against it, although there is certainly definite narrowing of these passages. The results of examination indicate that in these −A dogs the whole brain and probably the spinal cord is subjected to increased pressure. The cerebrospinal fluid mechanism tends to diffuse this rise of pressure to all parts of the central nervous system, but whether it succeeds in doing this in severe cases of −A deficiency or whether it fails in such instances because of a break in continuity of the fluid is not known. Where the anatomical changes above described are great, it is undoubted that the total capacity of the spaces containing cerebrospinal fluid is much reduced as compared with that of the normal animal. It might be expected therefore that the one known function of this fluid, namely to distribute pressure evenly and thereby to lower it at local points of excessive pressure, as for instance in the posterior fossa of the cranium, is impaired in −A animals.

C. *Vertebral column, spinal cord and spinal nerves*

The kind of differences observed in the shape and texture of the bones of the skull of −A and +A dogs can be seen also in the bones of the vertebral column. All delicacy of outline present in these complicated structures when normal disappears in the vitamin A deficient animals and the bones become swollen and coarse. There seems to be little or no increase in the overall dimensions of each vertebra, but all the processes, including the arches and articular processes, are thickened to a greater or less extent. For instance, the wings of the atlas vertebra in one animal which received vitamin A were 2·2 mm. thick, whereas the thickness of the same region in its litter mate not receiving vitamin A was 4·95 mm. Instead of finely moulded edges (Pl. IV, fig. 5*a*), bulbous protuberances are usually found (Pl. IV, fig. 5*b*). It is unnecessary to describe these anatomical changes in individual vertebrae here, but their effect on the spinal cord and the spinal roots calls for some consideration. When the spinal cord is exposed by the cutting through of the vertebrae, it can be seen at once to be much more closely packed into the spinal

canal in animals which were fed on −A diets than in those with a sufficiency of the vitamin. A fatty material can also be seen in the former closely enveloping the cord between the dura and the vertebrae. This probably does not indicate an actual increase in fat in this position, but may be the result of the reduced size of the space.

Some of the changes in the bones and tissues immediately surrounding the spinal cord are represented in Pl. V, figs. 6*a* and 6*b*, which are photomicrographs of sections through the intervertebral discs of the 5th cervical vertebra of litter mates, one a +A animal (Pl. V, fig. 6*a*) and the other a −A animal (Pl. V, fig. 6*b*). One of the difficulties in this work is that the bone overgrowths, especially in complicated structures, make histological examination of identical positions difficult or impossible. For instance, Pl. V, figs. 6*a* and 6*b* are both roughly through the centre of the dorsal root ganglion, but owing to the bone overgrowth, the relative positions of other structures are altered and are therefore not necessarily truly comparable. This difficulty is partially overcome in practice by examining the serial sections which have been made in most of these experiments, but it is not possible to publish the necessary photomicrographs to show this. It will be seen, however, that, although there is not much change in the total area covered by the cross-section of the bones in these two dogs, the internal space traversed by the spinal cord, its membranes and nerves is greatly reduced in the case of the −A dog. An idea of the size of the changes in the various areas of such cross-sections can be obtained from the following figures, which represent a series of measurements made by weighing paper which just covered each part of the section and estimating each area by the weight of paper. The animals were injected with Wittmaack's solution, which tends to prevent shrinkage, so that the figures obtained probably approximate to those in life.

Areas of various regions of vertebral cross-sections (5th cervical) of +A and −A dogs

Regions	Sq. cm. actual area		Percentage of area	
	+A	−A	+A	−A
Spinal cord	0·338	0·336	8·05	8·01
Space between cord and dura	0·212	0·160	5·05	3·81
Space between dura and bone	0·668	0·384	15·9	9·15
Intervertebral disc and body of vertebra	1·428	1·547	34·0	36·88
Lateral portions of vertebra	1·371	1·614	32·65	38·48
Whole section of vertebra	4·200	4·195	100·0	100·0

These figures show (1) that the total area covered by the cross-sections of the comparable bones is not significantly altered in the −A

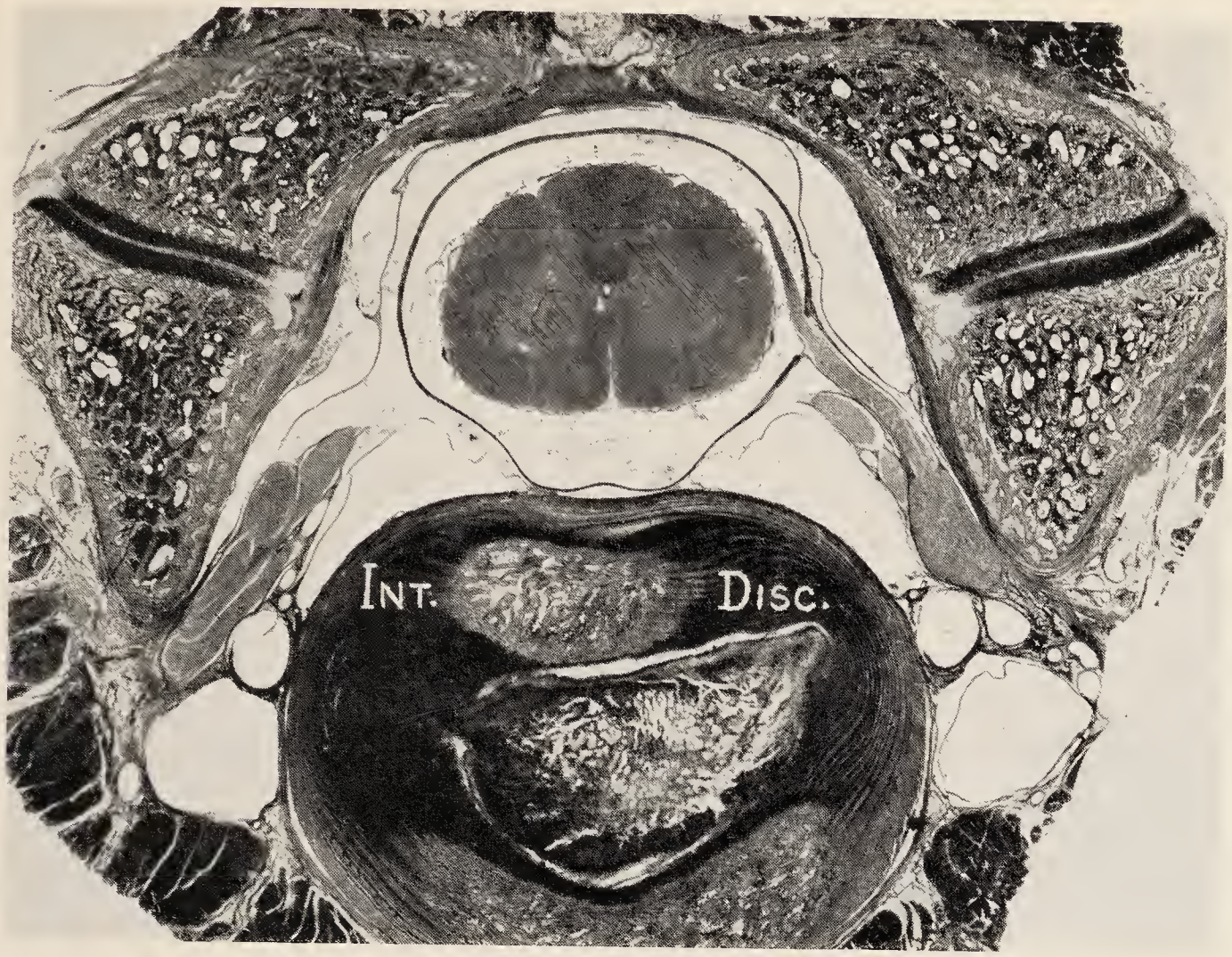

Fig. 6*a*.

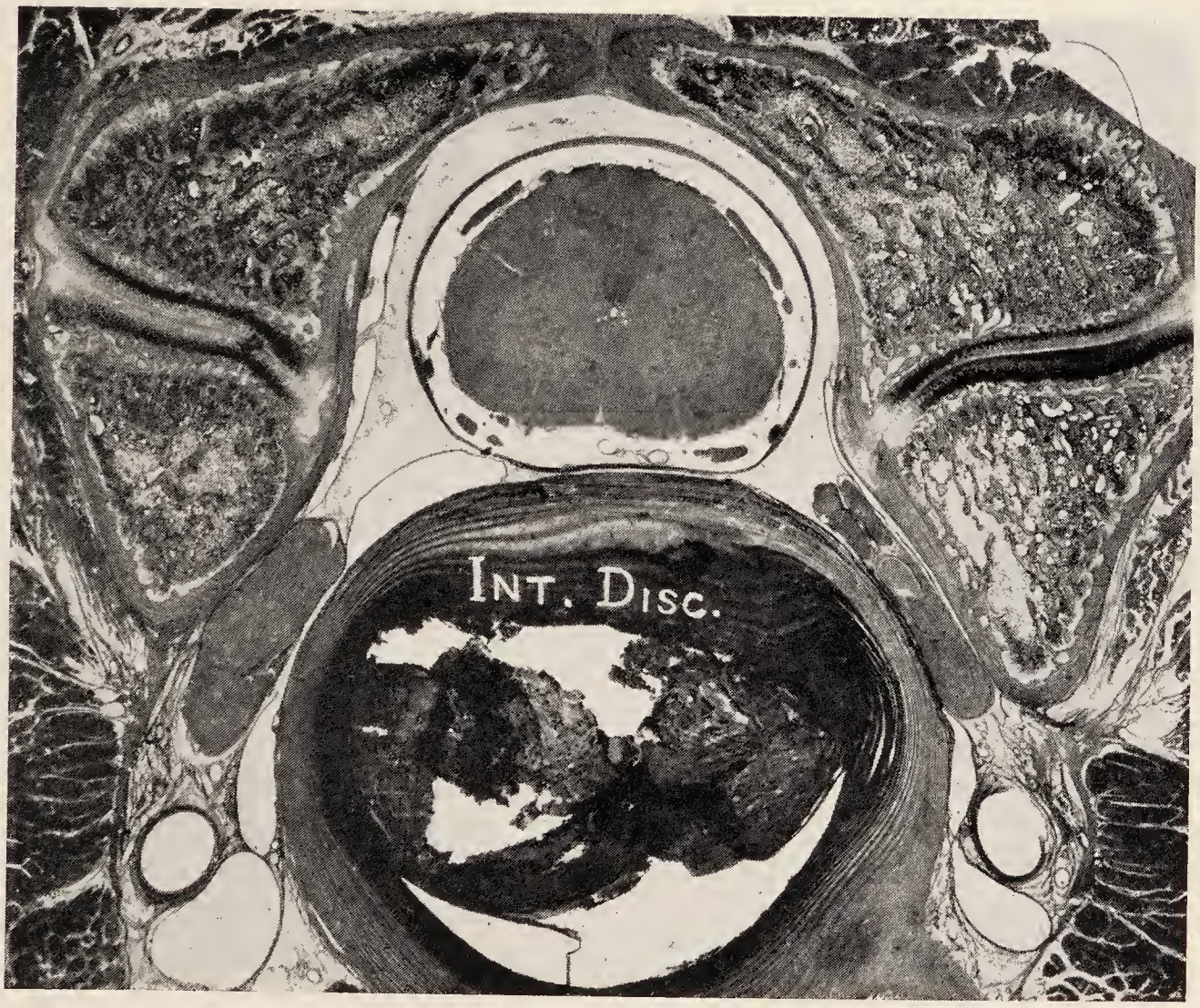

Fig. 6*b*.

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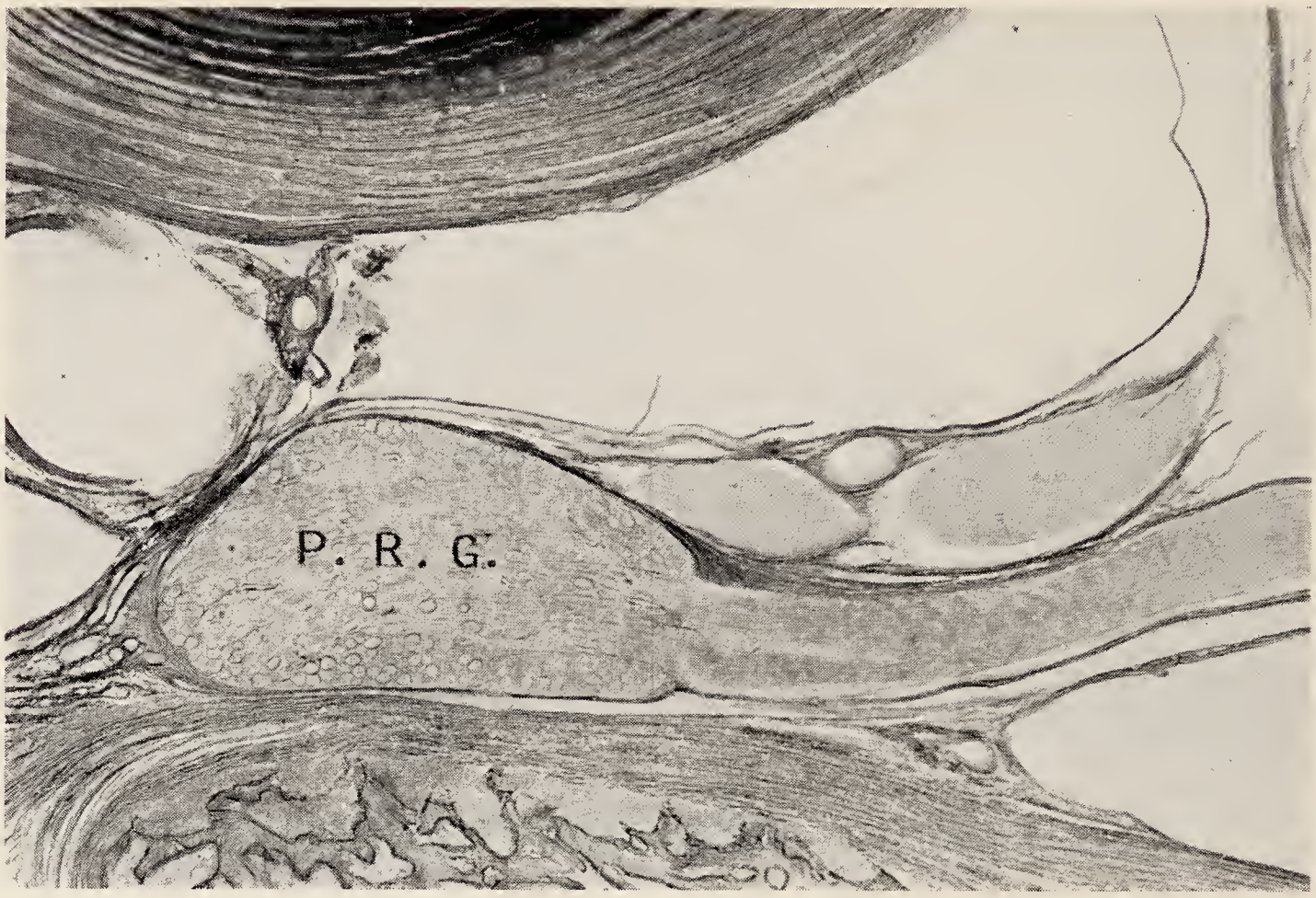

Fig. 7*a*.

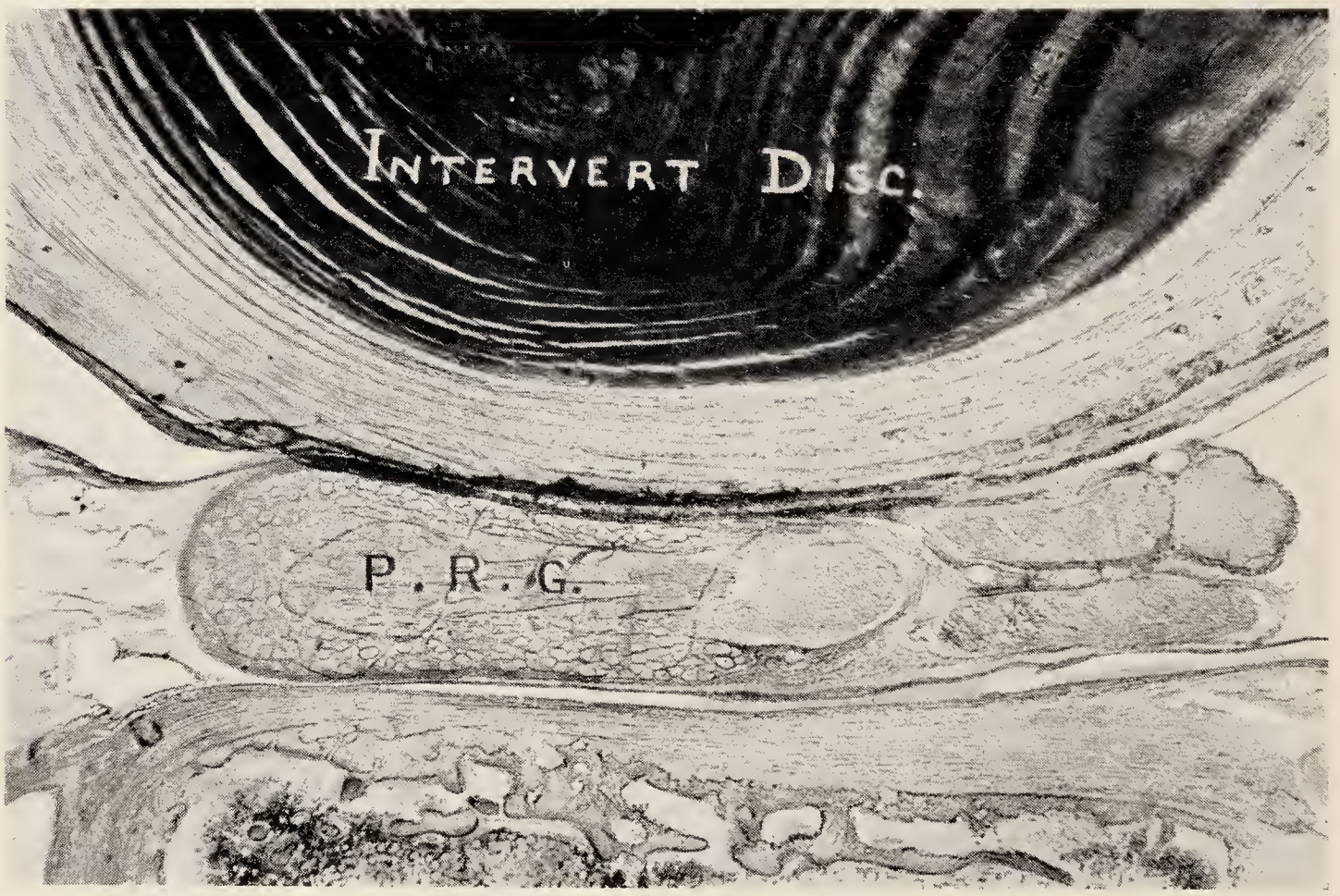

Fig. 7*b*.

animal, i.e. the vertebrae are not generally larger but are only locally thickened; (2) that the area of the lateral portions of the vertebra is increased in the −A dog; (3) that the main change in the −A dog is the reduction in space between the dura and the bone and to a less extent between the spinal cord and the dura.

Examination shows that one reason for the reduction in size of the spinal canal is the inward growth of the lateral mass of the vertebra, thus encroaching on the spinal canal.

The change in position of the vertebral artery and vein relative to the body of the vertebrae can also be seen. In the −A animal (Pl. V, fig. 6*b*) these vessels are placed more dorsally in relation to the intervertebral disc than those in the +A animal (Pl. V, fig. 6*a*).

As regards the space surrounding the spinal cord, it will be seen that (1) the reduction of the space between the dura mater and the cord is general but is greater on its ventral side, and the slight swelling in this position in the normal animal (Pl. V, fig. 6*a*) is absent from the −A animal (Pl. V, fig. 6*b*); (2) there is a great reduction in the size of the spaces between the body and the wings of the vertebrae for the passage of the spinal nerves. In fact, on the left side of the −A dog (Pl. V, fig. 6*b*) compression of these nerves and especially of the posterior root ganglion can be seen between the wings and the body of the vertebra: this state of compression, in comparison with the free space occupied by the corresponding nerves and ganglion in the normal animal, can be better appreciated in Pl. VI, figs. 7*a* and 7*b*, which are higher power photomicrographs. There is also some lengthening of the spinal nerves during this passage through the spinal canal of −A dogs, because the nerve roots pass more obliquely across the canal towards the periphery than in the +A animals.

The pressure exerted by the bones on the spinal nerves and the posterior root ganglion in the animals fed on vitamin A deficient diets is undoubtedly largely responsible for the degenerative changes previously described in these nerves [Mellanby, 1933, 1934, 1935], although at the time this was not appreciated. Attention, however, was drawn to what at that time seemed a curious fact, namely, that the anterior roots in the spinal canal central to the posterior root ganglia generally escaped degenerative changes, whereas the nerve fibres of the posterior roots in the same position were often degenerate. The reason for this difference in distribution of nerve degeneration now seems clearer. The nerve cells of the posterior root ganglion are often damaged by the effects of pressure and their axis cylinders, both peripheral and central to the ganglion,

therefore degenerate. On the other hand, even if the anterior roots are destroyed by pressure at the same spot, the fibres central to the lesion will escape degeneration since their ganglionic origin is in the anterior horns of the spinal cord. The fibres of the anterior spinal nerves peripheral to the compression may, however, be destroyed. There is certainly great degeneration in the sciatic nerve and other peripheral nerves of spinal origin, but whether the efferent as well as the afferent fibres are affected is not known.

Another observation made in the earlier work was that all the spinal nerves in a given $-$A dog did not suffer equally. Even at the same level of the vertebral canal, sometimes the spinal nerves on one side escaped destructive changes while those on the other side suffered. The reason for this difference can now be understood. In Pl. V, fig. 6*b*, for instance, it will be seen that the spinal nerves and posterior root ganglion of the right side (left side in Pl. V, fig. 6*b*) are not subjected to the same pressure effects as those of the left, so that the latter are more destroyed than the nerves of the right side.

It may be asked whether the bone overgrowth and malformation in the vertebrae of $-$A dogs explain all the degenerative changes seen in the cord. The bone abnormalities will certainly account for the degeneration of fibres having their origin in the posterior root ganglia and entering the cord. It has also been explained that the bone changes in the upper cervical region and in the bones surrounding the medulla oblongata are particularly large, so that the pressure effects in this region are great and may well be responsible for the increase in the number of degenerated fibres in the nervous system of these parts. Direct pressure on the cerebellum, medulla and upper cervical region of the cord may bring about the degenerative changes of the anterior and posterior cerebellar tracts which are so characteristic in these animals, although it is not easy to understand how this happens. It is even more difficult to explain these degenerations otherwise, for they are fibres of the second ascending neurone series and their degeneration cannot be accounted for by destruction of the posterior root ganglia of the spinal cord unless such destruction has affected the cells of the second neurone which, from our present knowledge, is unlikely. It is certainly of interest that much more nerve degeneration is found in these animals between the mid-brain and the 6th cervical vertebra, and it is in these parts that the bone overgrowth is larger and the mechanical distorting effects on the central nervous system in this position correspondingly greater.

While, therefore, it is not possible as yet to state categorically that

bone overgrowth of the type described can be held responsible for the degree and distribution of nerve degeneration both in the central and peripheral nervous systems in these animals, the evidence in favour of this view is strong. In later publications the subject will be dealt with from the point of view of the cranial nerves and the overgrowth of the bone surrounding these nerves, and here again it will be seen that the evidence favours the view that degeneration of these nerves is related directly to the bone change and indirectly also to the increased intracranial pressure resulting therefrom.

There is one aspect of this problem which has not been dealt with here, namely the effect of the bone overgrowth on the blood supply to the brain and spinal cord. This may be of some significance because it is undoubted that the bony channels through which the vascular supply of the nervous system is carried are often much constricted in these −A animals as compared with normal animals. How far the displacement and constriction of the blood vessels interfere with the nourishment of the nervous tissue has not been sufficiently worked out. The foramina and other places where the blood vessels pass through bones, although narrowed, do not appear to be completely closed in any case so far examined, and it may be that the blood supply remains adequate. The point requires further examination.

IV. How does vitamin A affect bone growth?

The gross effects of vitamin A deficiency on bone structure have been described and the question now to be considered is how these changes are produced, for with such knowledge it may be possible to determine the part played by vitamin A in normal bone growth.

A. *Histological examination*

In order to compare the histological appearance of bone tissue in +A and −A dogs, it is desirable to examine sections through identical parts of the same bones of litter mates. As previously mentioned, however, the complicated overgrowths of temporal and certain other bones make it difficult to obtain such sections. For this reason simple bones, i.e. femur shafts, have been used for comparison in this paper, although the changes are not so great as in the case of many other bones.

In comparing the cross-sections of the femur shafts of these +A and −A dogs, the most obvious differences are the thickening of the bone (see Pl. IV, figs. 8*a* and 8*b*) and the reduction in size of the marrow cavity in the −A dogs. In some cases the bones are also actually larger

in diameter, but this enlargement is not so common as the thickening of the bony layers. Whereas in the +A dogs the bones are comparatively compact, in the −A dogs they may be more or less compact towards the outer surface, but the greater part of the bone is cancellous in nature; the amount of this tissue depends on the extent of the deficiency and the length of the dieting period. The trabeculae of the cancellous bone generally run parallel with the outer surface.

If we now examine in more detail the sections represented in Pl. IV, figs. 8*a* and 8*b*, we see that in the normal dog the red marrow is found in a well-defined layer adjacent to the endosteal surface of the bone but clearly differentiated from it; there is no such marrow in the cavities of the bony layer. In the section from the −A dog, however, red bone marrow is diffuse and mixed up with the trabeculae in the inner third of the bony layer, while the outer two-thirds are free from it. If one of the trabeculae surrounded by red marrow is examined under a higher power, it will be seen that it is covered by a layer of active osteoblasts on the side nearer the marrow cavity, while on the outer side osteoblasts are reduced in number and not as active. The osteoclasts, however, are found mainly on the outer side of the trabeculae and judged from the size of the Howship's lacunae are more active when in that position. There are many more osteoclasts in the cancellous bone of the femur shafts of −A dogs than in the more compact bone of +A animals; for instance, in comparable sections of litter mates 380 osteoclasts were counted in a −A dog and only 144 in the corresponding +A animal. In these counts only cells adjacent to the bone were included, but it is interesting to note that the megakaryocytes were also increased in the −A animal.

Thus, while the more active osteoblasts on the endosteal side of the trabeculae lay down new bone, the osteoclasts on the outer side remove much of it. This probably accounts for the encroachment of cancellous bone on the marrow cavity in the −A animal and it seems from the material at present available to be the process by which the bone is converted from the compact to the cancellous state.

In addition to this effect of vitamin A deficiency on the osteoblasts and osteoclasts which are found in or near the red bone marrow, a similar effect can be seen on these cells in the subperiosteal region of bone. In this case also there is an increase in the activity of osteoblasts and probably also of osteoclasts, but the increase is not nearly so great as in the medullary bone. It follows, therefore, that the subperiosteal bone of −A dogs, although not so compact as in the normal animal, is much more compact than in the medullary two-thirds of the same bone.

The actual bone formed in the vitamin A deficient animal is probably not far removed from the normal bone and there is no increase of osteoid tissue. It is the gross arrangement of the bone which is so strikingly changed. It is probable, therefore, that the main effect of vitamin A on bone growth is to control the activity and number of osteoblasts and osteoclasts, primarily those associated with the bone marrow but also to a less degree the same cells in the subperiosteal region. In its absence these cells become more active, but the change seems to be one only in intensity and not in function, and the relative increase in these activities remains balanced, so that the total amount of calcified tissue does not greatly differ in the vitamin A deficient animal from that in one receiving this vitamin in abundance.

Although the effect of vitamin A deficiency on bone growth in these animals undoubtedly has a deeper significance than that described, the apparent mechanism seems to explain the bone changes.

B. *Chemical examination*

It has already been stated that the bone developed in vitamin A deficiency tends to be cancellous in those places where, with abundant vitamin A in the diet, it would have been compact. It is interesting to find on chemical analysis that the actual *amount* of calcium in corresponding bones of +A and −A animals is roughly the same. Taking an average amount of calcium in the femur shaft per 1000 g. of body weight in two groups of ten +A and ten −A animals, it was found that the +A group had 0·131 g., while the corresponding figure for the −A group was 0·134 g. In other words, under these experimental conditions, the thick cancellous bone of the −A animal contains little or no more calcium than the thinner more compact bone of the +A animal.

A second point of interest shown by chemical analysis is the relatively high fat content of the bones of some of these −A dogs as compared with that of similar bones of +A litter mates. This difference is particularly great in animals which have been sufficiently long on the diets to allow large bone changes in the −A dogs.

C. *The controlling effect of vitamin A on osteoblasts and osteoclasts*

It might be thought that the well-established fact that vitamin D influences bone formation would offer some kind of analogy as a guide in studying the vitamin A action on bony tissue. This is, however, not the case. The main function of the antirachitic vitamin is to promote calcification of bone: in its absence there is an excess of osteoid tissue, and the

mechanism of bone formation breaks down because this tissue remains largely uncalcified or is only slowly calcified. In other words, vitamin D stimulates a process—that of deposition in the matrix of the calcium salts essential for normal bone formation. Vitamin A, however, appears to influence the cellular elements involved in bone growth. Histological examination of the bone indicates that, unlike the stimulant calcifying action of vitamin D, the action of vitamin A is to limit and control. It has been described above how, in the absence of vitamin A, the activity of such cells as osteoblasts and osteoclasts becomes excessive.

This action of vitamin A deficiency on bone cells and structure must call to mind the earlier established action of vitamin A deficiency in epithelial hyperplasia. It is undoubted that a deficiency of this vitamin in some young animals causes characteristic changes in epithelium, the most common being hyperplasia of squamous epithelium, keratinization changes in such epithelium and, in some cases, metaplasia of columnar to squamous epithelium. Probably the fundamental change is overgrowth of epithelial cells of all kinds, keratinization and metaplasia being secondary to this overgrowth. These changes are more prominent in young rats than in other experimental animals and it is probable that this is the reason why it is widely accepted as the main pathological change produced by absence of vitamin A, since most nutritional research has been done on rats. Another reason why so much attention has been given to this phenomenon is that, in the rat at least, the epithelial changes are commonly associated with local infection, so that young rats nearly always develop multiple foci of infection when deprived of vitamin A. Other young animals such as rabbits and dogs, although not escaping these changes, do not seem to develop them to the same extent as rats. In rabbits and dogs stratified epithelium certainly becomes hyperplastic, but metaplasia is much rarer than in rats.

In the present work attention is drawn to another type of tissue which undergoes what may be the same fundamental change when vitamin A is deficient, namely excessive formation of osteoblasts and osteoclasts. It would appear then that the vitamin A not only controls the activity of epithelial cells but also of certain cells of mesoblastic origin. If this is established, it is obviously of some significance to cellular physiology in the mammal that there is a substance normally present in the body but not synthesized there, and provided of necessity in the food, whose main function is to control the number and degree of activity of certain epiblastic and mesoblastic cells. In its absence these cells increase abnormally in number and activity but do not, as do

cancer cells, change their function or act in a way different from the normal cells of the same type.

Summary

1. A function of vitamin A is to influence the structure of growing bones, probably by limiting the number and the degree of activity of osteoblasts and osteoclasts. In its absence from the growing dog osteoblastic and osteoclastic activity is increased, thus resulting in proliferation of cancellous at the expense of compact bone and causing many bones to lose their normally fine moulding and outline and to become thickened and enlarged.

2. Some of the main positions of bone overgrowth in the skull and vertebral column produced by the vitamin A deficient diets used in these experiments are described. These overgrowths are related to degenerative changes in the brain and in cranial and peripheral nerves, of which accounts have been given in earlier publications.

3. Overgrowth of the cranial bones may press on and produce deformity of parts of the brain. The greatest hypertrophy is found in the bones forming the posterior fossa of the skull, so that the medulla oblongata, pons, cerebellum and nerves in close association with these parts of the brain are more particularly affected. Most of the cranial nerves of young dogs are liable to be compressed and to suffer destructive changes if the A deficient diets are continued over long enough periods (about 4 to 8 months).

4. The posterior root ganglia and the anterior root nerves of the spinal cord may also be squeezed and the nerve fibres destroyed by overgrowth of the vertebral bones. The bone overgrowth and nerve degenerative changes are greatest in the cervical region of the cord.

5. In advanced cases on these vitamin A deficient diets there is evidence of a substantial increase in intracranial pressure. The pressure of the cerebrospinal fluid in the cisterna magna may be double that of animals receiving similar diets containing vitamin A. Associated with these changes the cisterna magna and the 4th ventricle are diminished in capacity, while a degree of internal hydrocephalus (expansion of the 3rd and lateral ventricles) indicates increased pressure, probably transmitted from the posterior fossa.

Note. Since this paper went to press, the author has seen a publication by L. A. Moore and J. F. Sykes (*Amer. J. Physiol.* [1940], **130**, 684) in which are described experiments showing increased cerebrospinal fluid pressure in calves on a ration deficient in vitamin A.

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EXPLANATION OF PLATES I–VI

Plate I

Figs. 2*a* and 2*b*. Mesial sections through foramen magnum of +A (*a*) and −A (*b*) dogs, to show reduced capacity of cisterna magna in −A dog (2*b*). Here again the intrusion of the cerebellum into the cisterna magna in 2*b* is seen.

Plate II

Figs. 3*a* and 3*b*. Coronal sections through 4th ventricles and lateral recesses (foramina of Luschka) of +A (*a*) and −A (*b*) dogs. Note the reduction in size, especially in dorso-ventral diameter of the 4th ventricle (IV), the packed appearance of the choroid plexus and the reduced diameter of the foramina of Luschka (Lu) of the −A dog (3*b*) as compared with the +A dog (3*a*).

Plate III

Figs. 4*a* and 4*b*. Coronal sections through the lateral (Lat.) and 3rd ventricles (III) of +A (*a*) and −A (*b*) dogs. Note the distension of these ventricles (internal hydrocephalus) in the −A dog (4*b*) as compared with the normal +A dog (4*a*).

Plate IV

Fig. 5*a*. Atlas vertebra of dog whose diet contained vitamin A. Note delicacy of outline and large vertebral canal.

Fig. 5*b*. Atlas vertebra of dog whose diet was deficient in vitamin A. Note bulbous appearance of all protuberances. Reduction in size of vertebral canal, but overall size of vertebra little affected.

Figs. 8*a* and 8*b*. Cross-sections of femur shafts of +A (*a*) and −A (*b*) dogs. (*a*) Showing nearly normal structure and (*b*) showing thickened cancellous shaft and great osteoclastic activity.

Plate V

Figs. 6*a* and 6*b*. Transverse sections through the 5th cervical vertebra and the spinal cords of +A (*a*) and −A (*b*) dogs. Note the reduction in size of the vertebral canal and the compression of the spinal root ganglion in the −A dog (6*b*) as compared with the +A dog (6*a*): also the alteration in shape of the dorsal surface of the intervertebral disc (Int. Disc.) in 6*b*.

Plate VI

Fig. 7*a*. Posterior root ganglion (P.R.G.) of dog receiving diet containing vitamin A.

Fig. 7*b*. Posterior root ganglion (P.R.G.) of dog receiving diet deficient in vitamin A. Note compression of ganglion between body and lateral processes of vertebrae.